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(58) Field of Search

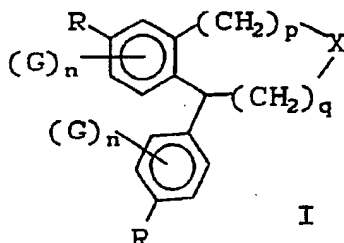
UK CL (Edition L) C2C CWE

INT CL⁵ C07C, C07D

ONLINE DATABASES: CAS ONLINE

(54) HIV integrase inhibitors

(57) Compounds of the general formula:



wherein

n is 0-3;

p is 1-2;

q is 1-2;

X is CH₂, O or N-R¹, and R¹ is H, C₁₋₄ alkyl or C₃₋₅ cycloalkyl;

R is

(a) C₁₋₆ alkyl;

(b) C₁₋₆ alkoxy;

(c) hydroxyl;

(d) halogen; (e) CN;

(f) NO₂;

(g) NHSO₂CH₃; or

(h) COOH;

G is H or R,

and salts or hydrates thereof have HIV integrase inhibiting activity.

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TITLE OF THE INVENTION

DIBENZYLHETEROCYCLIC OR DIBENZYLCHCLOALKYL DERIVATIVES
AS INHIBITORS OF HIV INTEGRASE

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BACKGROUND OF THE INVENTION

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A retrovirus designated human immunode-
ficiency virus (HIV) is the etiological agent of the
complex disease that includes progressive destruction
of the immune system (acquired immune deficiency
syndrome; AIDS) and degeneration of the central and
peripheral nervous system. This virus was previously
known as LAV, HTLV-III, or ARV. A common feature of
retrovirus replication is the insertion by
virally-encoded integrase of proviral DNA into the host
cell genome, a required step in HIV replication in
human T-lymphoid cells. Integration is believed to
occur in three stages: cleavage of two nucleotides
from the 3' termini of the linear proviral DNA;
covalent joining of the recessed 3' OH termini of the
proviral DNA at a staggered cut made at the host target
site; repair synthesis by host enzymes.

Nucleotide sequencing of HIV shows the presence of a pol gene in one open reading frame [Ratner, L. et al., Nature, 313, 277(1985)]. Amino acid sequence homology provides evidence that the pol sequence encodes reverse transcriptase, an integrase and an HIV protease [Toh, H. et al., EMBO J. 4, 1267 (1985); Power, M.D. et al., Science, 231, 1567 (1986); Pearl, L.H. et al., Nature 329, 351 (1987)].

It is known that some antiviral compounds act as inhibitors of HIV and are effective agents in the treatment of AIDS and similar diseases, e.g., azidothymidine or AZT. Applicants demonstrate that the compounds of this invention are inhibitors of HIV integrase, probably by inhibiting its endonucleolytic cleavage activity rather than its binding function. The particular advantage of the present invention is highly specific inhibition of HIV integrase. The compounds of the present do not inhibit a variety of other protein-nucleic acid interactions, including enzymatic reactions involving HIV reverse transcriptase, mammalian topoisomerase I, mammalian topoisomerase II, Eco RI endonuclease, or mammalian polymerase II, as well as other related interactions, e.g., involving HIV TAT protein.

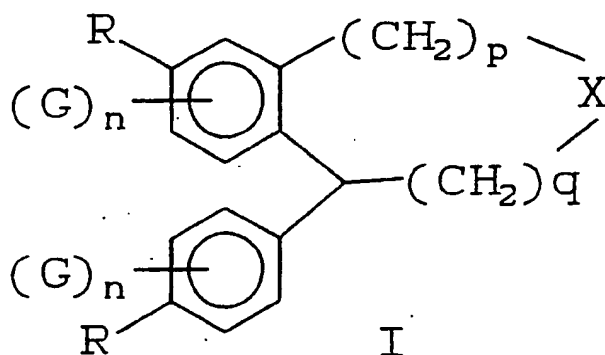
BRIEF DESCRIPTION OF THE INVENTION

Compounds of formula I, as herein defined, are disclosed. These compounds are useful in the inhibition of HIV integrase, the prevention of infection by HIV, the treatment of infection by HIV and in the treatment of AIDS and/or ARC, either as compounds, pharmaceutically acceptable salts or hydrates (when

appropriate), pharmaceutical composition ingredients, whether or not in combination with other antivirals, anti-infectives, immunomodulators, antibiotics or vaccines. Methods of treating AIDS, methods of preventing infection by HIV, and methods of treating infection by HIV are also disclosed.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

This invention is concerned with compounds of formula I, combinations thereof, or pharmaceutically acceptable salts thereof, in the inhibition of HIV integrase, the prevention or treatment of infection by HIV and in the treatment of the resulting acquired immune deficiency syndrome (AIDS). Compounds of formula I are defined as follows:



wherein

n is 0-3;

p is 1-2;

q is 1-2;

X is CH₂, O or N-R¹, and R¹ is H, C₁₋₄alkyl or C₃₋₅cycloalkyl;

R is (a) C₁₋₆ alkyl;
(b) C₁₋₆ alkoxy;
(c) hydroxyl;
(d) halogen;
5 (e) CN;
(f) NO₂;
(g) NHSO₂CH₃; or
(h) COOH;

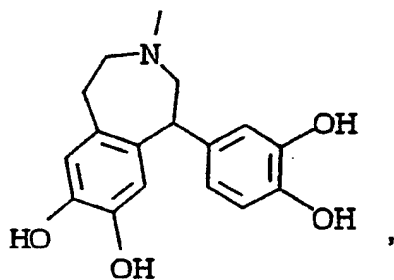
10 G is H or R,
or pharmaceutically acceptable salt or hydrate thereof.

A preferred compound of the present invention
is Compound A as follows:

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A:

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L-619, 323,

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7,8-Dihydroxy-1-(3,4-dihydroxyphenyl)-3-methyl-2,3,4,5-
tetrahydro-1H-3-benzazepine,

or pharmaceutically acceptable salt or hydrate thereof.

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The compounds of the present invention may have asymmetric centers and may occur, except when specifically noted, as racemates, racemic mixtures or as individual diastereomers, or enantiomers, with all isomeric forms being included in the present invention.

When any variable (e.g., G, R, etc.) occurs more than one time in any constituent or in formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

As used herein except where noted, "alkyl" is intended to include both branched- and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. "Halogen" or "halo" as used herein, means fluoro, chloro, bromo and iodo.

The compounds of the present invention can be synthesized by the following methods.

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SCHEME I

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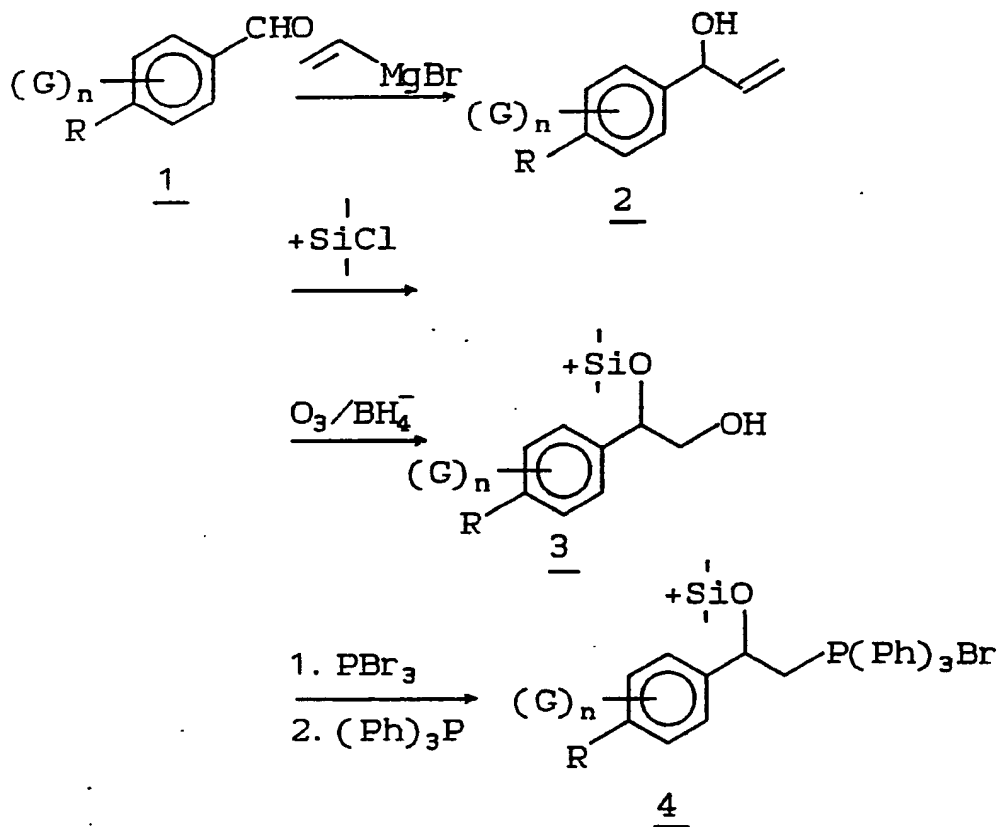
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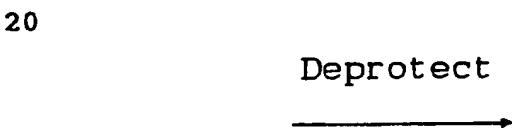
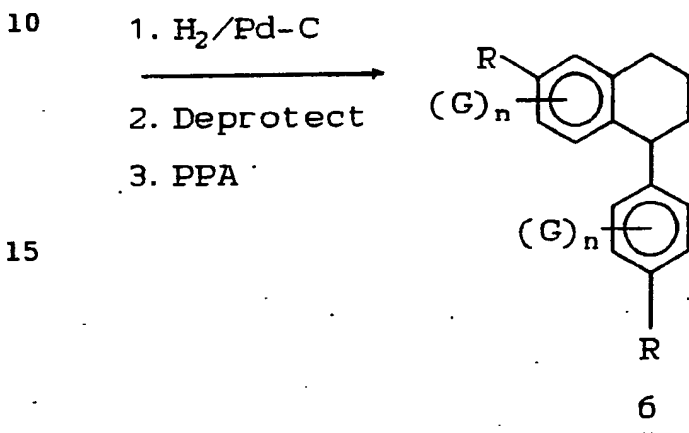
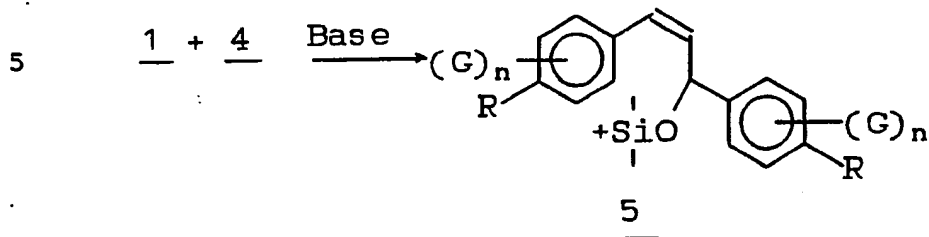
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One method of synthesizing the compounds of the present invention is found in Scheme I. The phenyl aldehyde reactant is treated with an alkylene Grignard reagent to give 2. Protection of the hydroxyl, followed by ozonolysis in the presence of a reducing agent affords 3. The Wittig approach can then be used, e.g. the aldehyde 1 is reacted with 4, which is a

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benzyl substituted with alkylene triphenyl phosphorane.
The resulting condensation product 5 yields an
unsaturated alkylene bridge, which is then hydrogenated,
deprotected, then subjected to acid-catalyzed
5 cyclodehydration, to yield 6. Further deprotection of
R or G groups may or may not be needed, as the case may
be. For example, OH groups protected with methyl
substituents may be dealkylated by reaction with
pyridine hydrochloride. It will be understood that the
10 appropriate protecting groups will be added to prevent
undesired side reactions with G or R substituents.

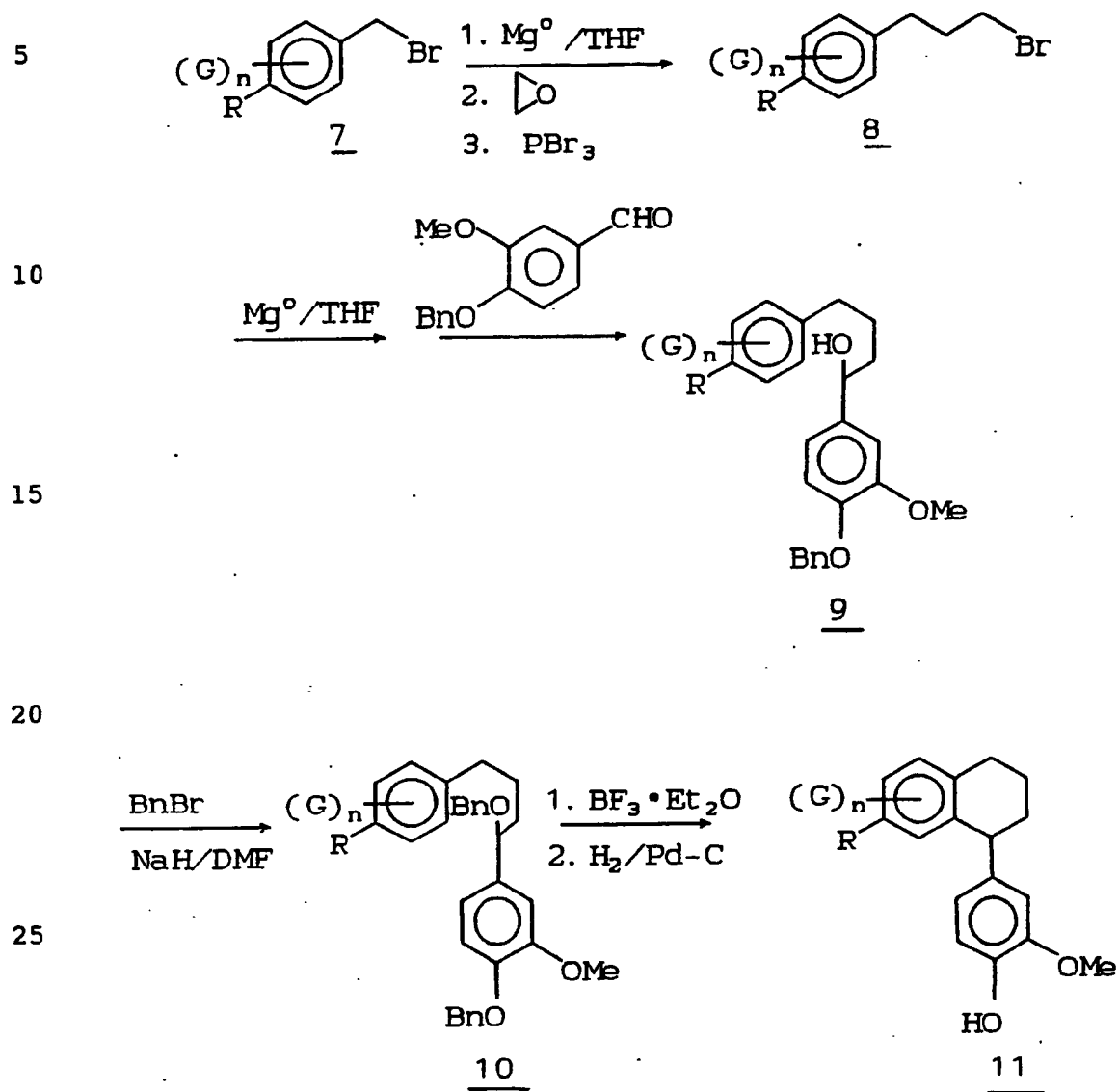
When $X=NR^1$ cyclodehydration is conducted with
phosphorus oxychloride in the Bischler-Napieralski
reaction instead of polyphosphoric acid (PPA). See
15 Examples 1-6, as well as Grethe, b. (ed.) Isoquinolines
Wiley New York 1981, pp. 142-161.

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25

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SCHEME II



Scheme II provides a method for synthesizing compounds of Formula I when $X=CH_2$. In Scheme II, formation of a Grignard reagent is performed by reaction of 7 with magnesium turnings. After reaction with oxirane, conversion of the resulting alcohol into the corresponding bromide 8 occurs by reaction with phosphorus tribromide. Preparation of a second Grignard reagent followed by reaction with the appropriate aromatic aldehyde, e.g. parabenzyl-
vanillinaldehyde, gives the alcohol intermediate 9. Formation of a good leaving group for cyclization is accomplished by, for example, benzylation with NaH as base, to afford 10. Intramolecular Friedel-Crafts cyclization in the presence of $BF_3 \cdot Et_2O$ followed by hydrogenation provides compounds of the present invention 11. For extensive discussion of synthetic routes related to Scheme II, see, for example, Boissin, P. et al., Tetrahedron 48, 687 (1992).

The compounds of the present inventions are useful in the inhibition of HIV integrase the prevention of treatment of infection by human immunodeficiency virus (HIV) and the treatment of consequent pathological conditions such as AIDS. Treating AIDS or preventing or treating infection by HIV is defined as including, but not limited to, treating a wide range of states of HIV infection: AIDS, ARC (AIDS related complex), both symptomatic and asymptomatic, and actual or potential exposure to HIV. For example, the compounds of this invention are useful in treating infection by HIV after suspected past exposure to HIV by e.g., blood transfusion, exchange of body fluids, bites, accidental needle stick, or exposure to patient blood during surgery.

For these purposes, the compounds of the present invention may be administered orally, parenterally (including subcutaneous injections, intravenous, intramuscular, intrasternal injection or
5 infusion techniques), by inhalation spray, or rectally, in dosage unit formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles.

Thus, in accordance with the present
10 invention there is further provided a method of treating and a pharmaceutical composition for treating HIV infection and AIDS. The treatment involves administering to a patient in need of such treatment a pharmaceutical composition comprising a pharmaceutical
15 carrier and a therapeutically-effective amount of a compound of the present invention.

These pharmaceutical compositions may be in the form of orally-administrable suspensions or tablets; nasal sprays; sterile injectable preparations,
20 for example, as sterile injectable aqueous or oleagenous suspensions or suppositories.

When administered orally as a suspension, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and
25 may contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents known in the art. As immediate release tablets, these compositions
30 may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art.

When administered by nasal aerosol or inhalation, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

The injectable solutions or suspensions may be formulated according to known art, using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

When rectally administered in the form of suppositories, these compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquidify and/or dissolve in the rectal cavity to release the drug.

The compounds of this invention can be administered orally to humans in a dosage range of 1 to 1000 mg/kg body weight in divided doses. One preferred dosage range is 0.1 to 100 mg/kg body weight orally in divided doses. Another preferred dosage range is 0.1 to 200 mg/kg body weight orally in divided doses. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a

variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

The present invention is also directed to combinations of the HIV integrase inhibitor compounds with one or more agents useful in the treatment of AIDS. For example, the compounds of this invention may be effectively administered, whether at periods of pre-exposure and/or post-exposure, in combination with effective amounts of the AIDS antivirals, immunomodulators, antiinfectives, or vaccines, such as those in the following table.

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TABLE

ANTIIVIRALS

| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|--------------------------------------|-------------------------------------|---|
| 5 | AL-721 | Ethigen (Los Angeles, CA) | ARC, PGL HIV positive, AIDS |
| | Recombinant Human Interferon Beta | Triton Biosciences (Alameda, CA) | AIDS, Kaposi's sarcoma, ARC |
| 10 | Acemannan | Carrington Labs (Irving, TX) | ARC (See also immunomodulators) |
| 15 | Cytovene | Syntex | sight threatening CMV |
| | Ganciclovir | (Palo Alto, CA) | peripheral CMV retinitis |
| 20 | d4T Didehydrodeoxy- thymidine | Bristol-Myers (New York, NY) | AIDS, ARC |
| | ddI Dideoxyinosine | Bristol-Myers (New York, NY) | AIDS, ARC |
| 25 | EL10 | Elan Corp, PLC (Gainesville, GA) | HIV infection (See also immunomodulators) |

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| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|--------------------------------------|---|---|
| | Trisodium Phosphonoformate | Astra Pharm. Products, Inc. (Westborough, MA) | CMV retinitis, HIV infection, other CMV infections |
| 5 | | | |
| | Dideoxycytidine; ddC | Hoffman-La Roche (Nutley, NJ) | AIDS, ARC |
| | | | |
| 10 | Novapren | Novaferon Labs, Inc. (Akron, OH) Diapren, Inc. (Roseville, MN, marketer) | HIV inhibitor |
| | | | |
| 15 | Peptide T Octapeptide Sequence | Peninsula Labs (Belmont, CA) | AIDS |
| | | | |
| 20 | Zidovudine; AZT | Burroughs Wellcome (Rsch. Triangle Park, NC) | AIDS, adv, ARC pediatric AIDS, Kaposi's sarcoma, asymptomatic HIV infection, less severe HIV disease, |
| 25 | | | neurological involvement, in combination with other therapies. |
| 30 | | | |

| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|---|--|--|
| 5 | Ansamycin IM 427 | Adria Laboratories (Dublin, OH) Erbamont (Stamford, CT) | ARC |
| 10 | Dextran Sulfate | Ueno Fine Chem. Ind. Ltd. (Osaka, Japan) | AIDS, ARC, HIV positive asymptomatic |
| | Virazole | Viratek/ICN | asymptomatic HIV |
| | Ribavirin | (Costa Mesa, CA) | positive, LAS, ARC |
| 15 | Alpha Interferon | Burroughs Wellcome (Rsch. Triangle Park, NC) | Kaposi's sarcoma, HIV in combination w/Retrovir |
| 20 | Acyclovir | Burroughs Wellcome | AIDS, ARC, asymptomatic HIV positive, in combination with AZT. |
| 25 | Antibody which neutralizes pH labile alpha aber- rant Interferon in an immuno- adsorption column | Advanced Biotherapy Concepts (Rockville, MD) | AIDS, ARC |

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| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|------------------|-----------------------|--|
| 5 | L-697,661 | Merck (Rahway, NJ) | AIDS, ARC, asymptomatic HIV positive, also in combination with AZT. |
| 10 | L-696,229 | Merck (Rahway, NJ) | AIDS, ARC, asymptomatic HIV positive, also in combination with AZT. |
| 15 | | | |
| 20 | L-735,524 | Merck (Rahway, NJ) | Aids, ARC, asymptomatic HIV positive, also in combination with AZT. |
| 25 | | | |
| 30 | | | |

IMMUNO-MODULATORS

| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|---|---|---|
| 5 | AS-101 | Wyeth-Ayerst Labs. (Philadelphia, PA) | AIDS |
| | Bropirimine | Upjohn (Kalamazoo, MI) | advanced AIDS |
| 10 | Acemannan | Carrington Labs, Inc. (Irving, TX) | AIDS, ARC (See also anti-virals) |
| 15 | CL246,738 | American Cyanamid (Pearl River, NY) Lederle Labs (Wayne, NJ) | AIDS, Kaposi's sarcoma |
| 20 | EL10 | Elan Corp, PLC (Gainesville, GA) | HIV infection (See also anti-virals) |
| 25 | Gamma Interferon | Genentech (S. San Francisco, CA) | ARC, in combination w/TNF (tumor necrosis factor) |
| 30 | Granulocyte Macrophage Colony Stimulating Factor | Genetics Institute (Cambridge, MA) Sandoz (East Hanover, NJ) | AIDS |

| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|--------------------|--------------------------|-------------------------------|
| | Granulocyte | Hoeschst-Roussel | AIDS |
| | Macrophage Colony | (Somerville, NJ) | |
| 5 | Stimulating Factor | Immunex (Seattle, WA) | |
| | Granulocyte | Schering-Plough | AIDS |
| | Macrophage Colony | (Madison, NJ) | |
| 10 | Stimulating Factor | | AIDS, in combination w/AZT |
| | HIV Core Particle | Rorer | seropositive HIV |
| | Immunostimulant | (Ft. Washington, PA) | |
| 15 | IL-2 | Cetus | AIDS, in combination |
| | Interleukin-2 | (Emeryville, CA) | w/AZT |
| | IL-2 | Hoffman-La Roche | AIDS, ARC, HIV, in |
| | Interleukin-2 | (Nutley, NJ) | combination |
| 20 | | Immunex | w/AZT |
| | Immune Globulin | Cutter Biological | pediatric AIDS, in |
| | Intravenous | (Berkeley, CA) | combination |
| 25 | (human) | | w/AZT |
| | IMREG-1 | Imreg | AIDS, Kaposi's |
| | | (New Orleans, LA) | sarcoma, ARC, PGL |
| | IMREG-2 | Imreg | AIDS, Kaposi's |
| 30 | | (New Orleans, LA) | sarcoma, ARC, PGL |

| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|--|--|---|
| | Imuthiol Diethyl Dithio Carbamate | Merieux Institute (Miami, FL) | AIDS, ARC |
| 5 | Alpha-2 Interferon | Schering Plough (Madison, NJ) | Kaposi's sarcoma w/AZT: AIDS |
| | Methionine- Enkephalin | TNI Pharmaceutical (Chicago, IL) | AIDS, ARC |
| 10 | MTP-PE Muramyl- Tripeptide | Ciba-Geigy Corp. (Summit, NJ) | Kaposi's sarcoma |
| 15 | Granulocyte Colony Stimulating Factor | Amgen (Thousand Oaks, CA) | AIDS, in combination w/AZT |
| 20 | rCD4 Recombinant Soluble Human CD4 | Genentech (S. San Francisco, CA) | AIDS, ARC |
| 25 | rCD4-IgG hybrids | | AIDS, ARC |
| | Recombinant Soluble Human CD4 | Biogen (Cambridge, MA) | AIDS, ARC |
| 30 | Interferon Alfa 2a | Hoffman-La Roche (Nutley, NJ) | Kaposi's sarcoma AIDS, ARC, in combination w/AZT |

| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|---|---|--|
| | SK&F106528 Soluble T4 | Smith, Kline & French Laboratories (Philadelphia, PA) | HIV infection |
| 5 | Thymopentin | Immunobiology Research Institute (Annandale, NJ) | HIV infection |
| 10 | Tumor Necrosis Factor; TNF | Genentech (S. San Francisco, CA) | ARC, in combina- tion w/gamma Interferon |
| | <u>ANTI-INFECTIVES</u> | | |
| 15 | | | |
| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
| | Clindamycin with Primaquine | Upjohn (Kalamazoo, MI) | PCP |
| 20 | Fluconazole | Pfizer (New York, NY) | cryptococcal meningitis, candidiasis |
| 25 | Pastille Nystatin Pastille | Squibb Corp. (Princeton, NJ) | prevention of oral candidiasis |
| | Ornidyl Eflornithine | Merrell Dow (Cincinnati, OH) | PCP |
| 30 | Pentamidine Isethionate (IM & IV) | LyphoMed (Rosemont, IL) | PCP treatment |

| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|--|---|---|
| | Piritrexim | Burroughs Wellcome (Rsch. Triangle Park, NC) | PCP treatment |
| 5 | Pentamidine isethionate for inhalation | Fisons Corporation (Bedford, MA) | PCP prophylaxis |
| 10 | Spiramycin | Rhone-Poulenc Pharmaceuticals (Princeton, NJ) | cryptosporidial diarrhea |
| 15 | Intraconazole- R51211 | Janssen Pharm. (Piscataway, NJ) | histoplasmosis; cryptococcal meningitis |
| | Trimetrexate | Warner-Lambert | PCP |

20

OTHER

| | <u>Drug Name</u> | <u>Manufacturer</u> | <u>Indication</u> |
|----|-------------------------------------|---|--|
| 25 | Recombinant Human Erythropoietin | Ortho Pharm. Corp. (Raritan, NJ) | severe anemia assoc. with AZT therapy |
| | Megestrol Acetate | Bristol-Myers (New York, NY) | treatment of anorexia assoc. w/AIDS |
| 30 | Total Enteral Nutrition | Norwich Eaton Pharmaceuticals (Norwich, NY) | diarrhea and malabsorption related to AIDS |

It will be understood that the scope of combinations of the compounds of this invention with AIDS antivirals, immunomodulators, anti-infectives or vaccines is not limited to the list in the above Table, but includes in principle any combination with any pharmaceutical composition useful for the treatment of AIDS.

The compound L-697,661 is an inhibitor of HIV reverse transcriptase and is 3-([4,7-dichloro-1,3-benzoxazol-2-yl)methylamino)-5-ethyl-6-methyl-pyridin-2(1H)-one or pharmaceutically acceptable salt thereof. The compound L-696,229 is an inhibitor of HIV reverse transcriptase and is 3-[2-(1,3-benzoxazol-2-yl)ethyl]-5-ethyl-6-methyl-pyridin-2(1H)-one or pharmaceutically acceptable salt thereof. The compound L-735,524 is an inhibitor of HIV protease and is N-(2(R)-hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4-(S)-hydroxy-5-(1-(4-(3-pyridyl-methyl)-2(S)-N'-(t-butylcarboxamido)-piperazinyl))-pentaneamide, or pharmaceutically acceptable salt thereof.

EXAMPLE 1

1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydro-isoquinoline

A stirred solution of N-(3,4-dimethoxyphenyl-ethyl)-3,4-dimethoxyphenylacetamide (5g, prepared according to Weisbach, J.A. *et al* J. Med. Chem. 11, 752 (1968) and Wiegrebbe, W., Archiv. Pharm. 297,362 (1964)), POCl₃ (2.5ml) in toluene (25 ml) was refluxed with stirring for 3 hours. The exotherm was cooled to room temperature, and diluted with petroleum ether (100 ml).

The supernatant was discarded, and the gummy residue washed with petroleum ether (2 x 50 ml). A volume of 100 ml ice water was added to the washed residue, the resulting mixture basified with conc. NH_4OH , and
5 extracted with CH_2Cl_2 (4 x 50 ml). The combined CH_2Cl_2 extracts were washed with H_2O (50 ml), sat. NaCl solution (50 ml) and dried (Na_2SO_4). After 1 hour the Na_2SO_4 was filtered off, the ether solvent removed, affording a viscous, amber oil (4.6 g).

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EXAMPLE 2

1-(3,4-dimethoxybenzoyl)-6,7-dimethoxy-3,4-dihydro-
isoquinoline

15

The product of Example 1 (4.6 g) was dissolved in ethanol (15 ml) and bubbled with air while stirring overnight. A pale yellow precipitate formed, which was filtered. The filtrate was washed (2 ml ethanol), dried and the residue chromatographed on
20 silica gel ($\text{CHCl}_3:\text{MeOH}$, 9:1). Yield: 0.9g, mp = 180-187°C. The filtrate was suspended in ethanol (15 ml) and O_2 bubbled with stirring overnight. About 0.65 g more of the pale yellow solid precipitated out, and was worked up as above. Total yield: 1.55 g.

25

EXAMPLE 3

1-(3,4-dimethoxybenzoyl)-6,7-dimethoxy-2-methyl-3,4-
dihydroisoquinolinium iodide

30

The ketone product of Example 2 (1.55 g, 4.4 mmol) was suspended in ethanol (25 ml), and heated to near reflux. Methylating agent CH_3I (1.25 g, 0.55 ml, 8.8 mmol) was added and the mixture refluxed with

stirring for 5 hours. An additional 0.25 ml of CH₃I was added to drive the reaction to completion and refluxing continued overnight. At 20 hours, the mixture was cooled to room temperature and filtered. The filtrate was a yellow solid, 1.5 g, mp = 178-180°C. An analytical TLC on silica gel (CHCl₃:MeOH, 9:1) confirmed nearly quantitative yield.

EXAMPLE 4

1-(3,4-dimethoxy- α -hydroxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride

To the ketone iodide product of Example 3 (1.4 g), suspended in MeOH (25 ml, not completely soluble), was added stepwise NaBH₄ (1 g). The mixture had vigorous gas evolution. It was refluxed with stirring in a steam bath for 2 hours, cooled to room temperature and the solvent removed. The residue was taken up in 35 ml water, extracted with CH₂CH₂ (4 x 50 ml). The CH₂CH₂ extracts were combined, washed with 50 ml of saturated NaCl solution and dried (Na₂SO₄). After filtering off Na₂SO₄, and removing solvent, a viscous, glassy residue was obtained (0.9 g). The residue was dissolved in ethanol/HCl, stirred, scratched to induce crystallization, and placed in an ice water bath. The resulting white solid was filtered and dried, to afford the title compound. mp = 218-221°C (dec.)

EXAMPLE 5

7,8-Dimethoxy-1-(3,4-dimethoxyphenyl)-3-methyl-
2,3,4,5-tetrahydro-1H-3-benzazepine HCl

5 A mixture of the product of Example 4 (14.3
g) in proprionic acid (190 ml) was warmed until the
mixture turned yellow. The mixture was heated to
reflux with vigorous stirring, then Zn dust (26.4 g)
10 was added gradually over about 10 minutes. The reflux
continued with vigorous stirring for 7 hours. A
resulting white solid was filtered and washed with
proprionic acid. The filtrate and washings were
combined and dried, taken up in water (200 ml) and
combined with a 50 ml H₂O wash of the white solid, to
15 give a combined liquid wash. The combined liquid wash
was basified with 10% NaOH, extracted with ether (4 x
250 ml). The combined ether extracts were washed with
water (200 ml), sat. NaCl solution (200 ml) and dried
(Na₂SO₄), to give a yellow oil (11.6 g). A white solid
20 crystallized in EtOH/HCl, mp = 196°-198°C.

Anal Calcd. for C₂₂H₂₇NO₄

C, 64.03; H, 7.16; N, 3.56.

Found C, 63.77; H, 7.01; N, 3.37.

25

EXAMPLE 6

7,8-Dihydroxy-1-(3,4-dihydroxyphenyl)-3-methyl-2,3,4,5-
tetrahydro-1H-3-benzazepine hydrobromide sesquihydrate,
a hydrate of Compound A

30

The methoxy product of Example 5 (2 g, 5.1
mmol) was dissolved in CH₂Cl₂ (200 ml) and cooled in a
dry ice-acetone bath. Boron tribromide (2.4 ml in 50
ml CH₂Cl₂) was added dropwise over 5 minutes, and the

Annealing and Klenow Labeling of Integrase Substrate Oligonucleotide

- 20 1. HIV integrase substrate oligonucleotide (SEQ ID
No:1) has been gel purified and is at a
concentration of 0.65 OD/ml (21.45 µg/ml), or 1 µg
= 46.6 µl
- 25 2. Lyophilize 1 µg of substrate oligo (SEQ. ID NO:1).
3. Resuspend in 48, µl dH₂O,
add 1 µl 1M Tris-HCl pH 7.5
and 1 µl 5M NaCl.
- 30 4. Heat at 85°C for 15 min, take heating block out of
heating unit and allow to slow cool on the bench
top to room temperature, usually for 1.5 to 2

hours, affording annealed oligonucleotide (SEQ. ID No. 1), which forms a hairpin loop.

5. Divide into 5 μ l aliquots and store at -20°C .

6. Prepare fresh 10X Klenow buffer:

500 μ l 1M Tris-HCl pH 7.2

100 μ l 1M MgCl_2

50 μ l 5M NaCl

10 1 μ l 1M DTT

349 μ l dH_2O

1000 μ l

7. Klenow reaction mix:

15 4 μ l dH_2O

2 μ l 10X Klenow buffer

5 μ l annealed oligonucleotide

5 μ l ^{32}P -dGTP

2 μ l 1mM TTP

20 2 μ l Klenow enzyme (5 Units/ μ l)

20 μ l

Incubate at room temperature in a plexiglass shielded box for 2 hours.

25

8. Add 30 μ l 20mM Tris-HCl pH 7.5, 100mM NaCl, 10mM EDTA buffer to the mix to stop the reaction.

30

9. Extract with 50 μ l of Phenol/chloroform (1:1).

10. Extract with 50 μ l of Chloroform/isoamyl alcohol (24:1).

11. The reaction mix is now ready for molecular sieve chromatography. It is stored at -20°C until chromatography can be done.

5 12. TCA precipitate aliquots coming off the column, then count both pellet and supernatant. Pool and save fractions having low counts in supernatant and high counts in the pellet, which contain labeled integrase substrate oligonucleotide.

10

HIV INTEGRASE ASSAY: TCA PRECIPITATION

1. On day of assay, pipet 5 μl of sample or 10% DMSO for controls into tubes kept on ice.

15

2. Prepare fresh reaction buffer on day of assay, and dispense eight (8) aliquots into tubes on ice for dispensing with multipipettor. Pipet 11 μl per tube.

20

Reaction buffer: 20 μl 1M Tris-HCl pH 7.8
3 μl 1M MnCl_2
3.5 μl 1.4 M Beta-mercaptoethanol (1:10 dilution of commercial stock solution)
5 μl 10 mg BSA/ml
518.5 μl dH_2O
550 μl , enough for 50 reactions

25

30

3. Thaw 1 tube of pooled integrase on ice.

4. Thaw labeled integrase substrate oligonucleotide, allow approx. 10000 cpm per reaction, dilute if necessary with 20mM Tris-HCl pH 7.5, 100 mM NaCl, 0.1 mM EDTA. Pipet 2 μ l of substrate solution to be used in the assay into 208 μ l of the dilution buffer, and sample 150 μ l into one polypropylene tube, place on ice until end of assay (=input counts). Pipet eight aliquots into tubes on ice for dispensing with multipipettor.
5. Pipet 2 μ l of HIV integrase enzyme [expressed in *E. coli* BL21(DE3) as described in LaFemina, R. et al., *J. Virol.* 65, 5624(1991)] into all reaction tubes except No Enzyme Control tubes. No Enzyme Control tubes receive 2 μ l each HA Buffer B (50mM Tris-HCl pH 7.5, 10% glycerol, 1mM DTT, 0.1mM EDTA, and 1M NaCl). Immediately add 2 μ l of substrate to all tubes.
6. Finger tap to mix gently. Place tubes into prewarmed racks in 37°C water bath. Incubate 60 min.
7. Add 5 μ l of 1 mg tRNA/ml solution to each tube, followed by 185 μ l of 11% cold TCA. Vortex hard. Place on ice at least 60 min.
8. Filter through polyvinylidene difluoride microporous filters to trap undesired TCA precipitate.
9. Determine average cpm of No Enzyme Controls (background). Determine average cpm of Enzyme Controls (100 % value). Subtract background from

all samples, divide by Enzyme control value,
multiply by 100, and subtract from 100% to
determine % Inhibition.

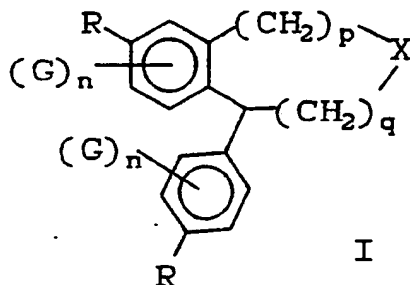
5 HIV Integrase Assay: Gel Cleavage

 Gel cleavage was conducted according to
LaFemina, R. et al., J. Virol. 65, 5624(1991), as
follows. A 5'-end-labeled oligonucleotide representing
10 the terminal 20 nucleotides of the U5 LTR plus strand
(SEQ ID NO:2) was annealed with its complement (SEQ ID
NO:3) and 1 ng of the resulting radiolabelled substrate
was incubated with or without inhibitor for 1 h at 37°C
with 80 ng (1 µl) of HIV integrase purified from E.
15 coli BL21(DE3)/pET3c. The reaction was in 20 µl of 10
mM Tris-HCl (pH 7.8)-5 mM 2-mercaptoethanol containing
3 mM MnCl₂. Products were analyzed by electrophoresis
on 20% sequencing gels. The positions and sizes of the
substrate, 20 nucleotides, and the primary cleavage
20 product, 18 nucleotides, are readily determined. To
assay inhibition of HIV integrase, the reaction is
conducted with inhibitor having various concentrations
in the range of 0.1-10µg/ml. Compound A shows
detectable inhibition as low as 0.5 µg/ml.

25 While the foregoing specification teaches the
principles of the present invention, with examples
provided for the purpose of illustration, it will be
understood that the practice of the invention
encompasses all of the usual variations, adaptations,
30 or modifications, as come within the scope of the
following claims and its equivalents.

WHAT IS CLAIMED IS:

1. A compound of the formulas:



15 wherein

n is 0-3;

p is 1-2;

q is 1-2;

X is CH₂, O or N-R¹, and R¹ is H, C₁₋₄ alkyl
20 or C₃₋₅ cycloalkyl;

R is (a) C₁₋₆ alkyl;

(b) C₁₋₆ alkoxy;

(c) hydroxyl;

25 (d) halogen;

(e) CN;

(f) NO₂;

(g) NHSO₂CH₃; or

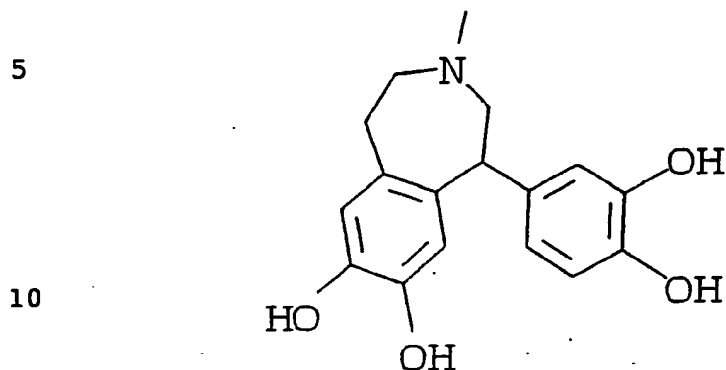
(h) COOH;

30

G is H or R,

or pharmaceutically acceptable salt or hydrate
thereof.

2. The compound



or pharmaceutically acceptable salt or hydrate thereof.

15

3. A method of inhibiting HIV integrase, comprising administering to a mammal an effective amount of a compound of any of Claims 1 or 2.

20

4. A method of preventing infection of HIV, or of treating infection by HIV or of treating AIDS or ARC, comprising administering to a mammal an effective amount of a compound of any of Claims 1 or 2.

25

5. A pharmaceutical composition useful for inhibiting HIV integrase, comprising an effective amount of a compound of any of Claims 1 or 2, and a pharmaceutically acceptable carrier.

30

6. A pharmaceutical composition useful for preventing or treating infection of HIV or for treating AIDS or ARC, comprising an effective amount of a compound of any of Claims 1 or 2, and a pharmaceutically acceptable carrier.

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|---|---|
| Relevant Technical Fields (i) UK Cl (Ed.L) C2C CWE (ii) Int Cl (Ed.5) C07C, C07D | Search Examiner P N DAVEY |
| | Date of completion of Search 8 NOVEMBER 1993 |
| Databases (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLINE DATABASES: CAS ONLINE | Documents considered relevant following a search in respect of Claims :- 1-6 |

Categories of documents

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|----------|--|----------------------|
| X | GB 1574208 (SMITHKLINE) see eg examples 5, 8 and 11 | 1 at least |
| X | GB 1486001 (BEECHAM) see eg examples 24 and 25 | 1 at least |
| X | GB 1335261 (HOFFMANN-LA ROCHE) see examples 5, 6, 15 and 16 | 1 at least |
| X | EP 0391554 A1 (UNIV PENNSYLVANIA) see eg table 3 and examples 3, 4, 6 and 7 | 1 at least |
| X | EP 0286293 A1 (YAMANOUCHI) see eg reference. Examples 11 and 18 and examples 2, 3, 6, 7 and 11 | 1 at least |
| X | EP 0244088 A2 (SMITHKLINE BECKMAN) see eg cpd 11 and example 8 | 1 at least |
| X | EP 0087319 A1 (SMITHKLINE BECKMAN) see eg examples 1 and 2 | 1 at least |
| X | US 4265889 (SMITHKLINE) see eg example 4 | 1 at least |

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